National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2387

Peanut Butter

This Standard Reference Material (SRM) is intended primarily for use in validating methods for determining proximates, fatty acids, calories, vitamins, elements, amino acids, aflatoxins, and acrylamide in peanut butter and similar matrices. This SRM can also be used for quality assurance when assigning values to in-house control materials. A unit of SRM 2387 consists of three jars of peanut butter containing 170 g each.

Certified Concentration Values: The certified concentration values of fat, selected fatty acids, elements, and tocopherols in SRM 2387 are provided in Tables 1 and 2. Values were derived from the combination of results provided by NIST and collaborating laboratories. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or accounted for by NIST [1]. The certified values in this material are the equally weighted means of the mean NIST result and the mean of the measurements made by collaborating laboratories; the associated uncertainties are expanded uncertainties at the 95 % level of confidence [2,3]. Values are reported on an as-received (not dry-mass) basis in mass fraction units [4].

Reference Concentration Values: Reference concentration values for additional proximates, fatty acids, amino acids, calories, total dietary fiber, vitamins, aflatoxins, and acrylamide are provided in Tables 3 through 7. Reference values are noncertified values that are the best estimate of the true values based on available data; however, the values do not meet the NIST criteria for certification [1] and are provided with associated uncertainties that may reflect only measurement reproducibility, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods.

Expiration of Value Assignment: The value assignment of this SRM is valid until **31 December 2009**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate. Value assignment is nullified if the SRM is damaged, contaminated, or modified.

Maintenance of SRM Value Assignment: NIST will monitor this SRM over the period of its value assignment. If substantive technical changes occur that affect the value assignment before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

Coordination of the technical measurements leading to the certification of this SRM was performed by K.E. Sharpless of the NIST Analytical Chemistry Division and H.B. Chin, I-P. Ho, and D.W. Howell of the National Food Processors Association (NFPA, Dublin, CA and Washington, DC).

Analytical measurements at NIST were performed by C.S. Phinney, K.E. Sharpless, and L.J. Wood of the NIST Analytical Chemistry Division. Analyses for value assignment were also performed by the laboratories listed in Appendices A through C.

Statistical analysis was provided by J.H. Yen of the NIST Statistical Engineering Division.

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Certificate Issue Date: 29 September 2004
See Certificate Revision History on Page 8

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The support aspects involved with the certification and issuance of this SRM were coordinated through the NIST Standard Reference Materials Program by J.C. Colbert and B.S. MacDonald of the NIST Measurement Services Division.

NOTICE AND WARNING TO USERS

Storage: The peanut butter should be frozen at –20 °C until required for use.

WARNING: For laboratory use only. **NOT** for human consumption.

INSTRUCTIONS FOR USE

Prior to removal of a test portion for analysis, a jar of peanut butter should be thawed under refrigeration overnight. The contents of a jar should be mixed thoroughly prior to removal of a test portion. Test portions used for NIST analyses described below were: 5 g to 7 g for tocopherols; 1 g for fat and fatty acids; and 0.5 g for elements.

PREPARATION AND ANALYSIS

Preparation: SRM 2387 is creamy peanut butter containing roasted peanuts, sugar, partially hydrogenated vegetable oils (48 % rapeseed, 40 % cottonseed, and 12 % soybean oil), and salt, and was prepared for NIST as part of a larger production run. Raw, shelled Florunner (primarily) peanuts were received from several suppliers and were roasted. The skins were removed from the roasted peanuts, and discolored peanuts were discarded. The roasted peanuts were then ground, and the remaining ingredients were added. After mixing, the peanut butter was further ground to a fine particle size, air was removed, and the peanut butter was cooled and packed in colorless polyethyl tetraethylene (PETE) jars with white screw caps and foil liners.

NIST Analyses for Fat: One set of three samples of peanut butter was prepared for gravimetric analysis of fat. One-gram portions of peanut butter were mixed with diatomaceous earth. The mixture was then briefly chilled at 4 °C to improve handling. The fat was then extracted from the mixture by pressurized fluid extraction (PFE) using hexane:acetone (4:1 volume fraction). Extracts were evaporated under nitrogen and then dried at 100 °C to constant mass.

NIST Analyses for Fatty Acids: Twelve fatty acids were measured in two sets of six samples of peanut butter prepared on two different days. The fat was extracted from approximately 1 g samples of peanut butter by PFE using a mixture of hexane:acetone (4:1 volume fraction). Methyl nonadecanoate (C19:0 fatty acid methyl ester [FAME]) was used as an internal standard. A two-step process employing methanolic sodium hydroxide and boron trifluoride was used to convert the fatty acids to their methyl esters. FAMEs were extracted into hexane and analyzed by gas chromatography with flame ionization detection.

NIST Analyses for Elements: Calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc were measured in eight jars of peanut butter. Two 0.5 g portions were taken from each jar and digested in a nitric, perchloric, and hydrofluoric acid mixture. Because of the high fat content, the samples were predigested on a hotplate before digestion in a microwave oven. Digests were transferred to plastic bottles and diluted with the appropriate volume of 1.5 % (volume fraction) nitric acid. To correct for matrix effects caused by differences between samples and calibrants, the method of standard additions was used; spikes were added to one aliquot prepared from each 0.5 g test portion. Four measurements using inductively coupled plasma optical emission spectrometry (ICP-OES) were made and averaged for each sample and each spiked solution. Results were corrected for spike recoveries.

NIST Analyses for Tocopherols: δ-Tocopherol, γ- (plus β-) tocopherol, and α-tocopherol were measured in test portions taken from six jars of peanut butter over a seven-day period. (The peanut butter may contain β-tocopherol, but the chromatographic system described below is incapable of resolving β- and γ-tocopherol; the instrument was calibrated using only γ-tocopherol.) Samples of approximately 5 g to 7 g were saponified using potassium hydroxide. Analytes were extracted into a mixture of diethyl ether and hexane, which was subsequently evaporated, and the analytes were redissolved in a mixture of ethanol and ethyl acetate. Samples were analyzed by liquid chromatography (LC) on a C_{18} column; analytes were eluted using a gradient of acetonitrile, methanol, and ethyl

acetate [5]. A programmable UV/visible absorbance detector set to 450 nm for measurement of *trans*-β-apo-10'-carotenal oxime (the internal standard) and a fluorescence detector (excitation wavelength of 295 nm, emission wavelength of 335 nm) were used for quantitation of the tocopherols.

Analyses by Collaborating Laboratories: Data from three additional sources were used for certification of this material: an interlaboratory comparison exercise organized by the NFPA Food Industry Analytical Chemists Subcommittee (FIACS) with 13 laboratories participating (Appendix A); four laboratories participating in an exercise in which only aflatoxins were measured (Appendix B); 17 laboratories participating in an exercise organized by the Joint Institute for Food Safety and Applied Nutrition (JIFSAN) Acrylamide Working Group in which acrylamide was measured (Appendix C). Not every laboratory measured every analyte. The laboratories listed in Appendix A were asked to use AOAC methods or their equivalent, to make single measurements from each of two jars, and to report the analytical method that was used. The laboratories listed in Appendix B were asked to use their usual methods to make single measurements of aflatoxins in each of three jars. The laboratories listed in Appendix C were asked to use their usual methods to make duplicate measurements of acrylamide in a single jar. A summary of the methodological information and the number of laboratories using a particular analytical technique is provided in Appendix D. The methods used by NIST are included in this listing as well.

Homogeneity Assessment: The homogeneity of calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, zinc, fatty acids, and tocopherols was assessed at NIST using the methods described above. A small but statistically significant heterogeneity was found for some analytes, and an inhomogeneity component of approximately 1 % has been included in the expanded uncertainty for all analytes.

Value Assignment: The laboratories listed in Appendix A reported values for 2 to 12 analyses. The laboratories listed in Appendix B reported values for three to nine analyses. The laboratories listed in Appendix C reported values for two to three analyses. The mean for each laboratory was determined from these values, and a mean of laboratory means was calculated. In cases where NIST also made measurements, this mean of means was averaged with the NIST mean to obtain the assigned value. In cases where NIST did not make measurements, the mean of laboratory means became the assigned value.

Table 1. Certified Concentration Values for Fat and Selected Fatty Acids[a]

	Mass Fraction (%)
Fat (Extractable)	51.6 ± 1.4
Fat (Sum of Fatty Acids)[b]	49.8 ± 1.9
Saturated Fat[c]	10.4 ± 0.2
Monounsaturated Fat[c]	24.4 ± 0.9
Polyunsaturated Fat[c]	13.2 ± 0.4

	Mass Fraction (%) as the Triglyceride	Mass Fraction (%) as the Fatty Acid
Tetradecanoic Acid (C14:0) (Myristic Acid)	0.025 ± 0.002	0.024 ± 0.002
Hexadecanoic Acid (C16:0) (Palmitic Acid)	5.18 ± 0.15	4.94 ± 0.15
(Z)-9-Hexadecenoic Acid (C16:1 n-7) (Palmitoleic Acid)	0.046 ± 0.011	0.044 ± 0.010
Octadecanoic Acid (C18:0) (Stearic Acid)	2.23 ± 0.08	2.13 ± 0.08
(Z)-9-Octadecenoic Acid (C18:1 n-9) (Oleic Acid)	24.43 ± 0.94	23.38 ± 0.90
(Z)-11-Octadecenoic Acid (C18:1 n-7) (Vaccenic Acid)	0.266 ± 0.017	0.255 ± 0.016
(Z,Z)-9,12-Octadecadienoic Acid (C18:2 n-6) (Linoleic Acid)	13.75 ± 0.43	13.15 ± 0.41
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3 n-3) (Linolenic Acid)	0.031 ± 0.001	0.030 ± 0.001
Eicosanoic Acid (C20:0) (Arachidic Acid)	0.739 ± 0.030	0.710 ± 0.029
(Z)-11-Eicosenoic Acid (C20:1 n-9) (Gondoic Acid)	0.669 ± 0.032	0.643 ± 0.031
Docosanoic Acid (C22:0) (Behenic Acid)	1.88 ± 0.08	1.81 ± 0.08
Tetracosanoic Acid (C24:0) (Lignoceric Acid)	0.808 ± 0.045	0.781 ± 0.044

[a] Each certified concentration value for fat and individual fatty acids, expressed as a mass fraction on an as-received basis, is the weighted mean of results provided by the laboratories listed in Appendix A and NIST. The uncertainty in the certified values, calculated according to the method described in the ISO and NIST Guides [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory, within-laboratory, and inhomogeneity components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. Analytical methodology information is provided in Appendix D. Some fatty acid data from collaborating laboratories were excluded if the laboratory's mean result was more than three standard errors from the mean of the interlaboratory comparison exercise.

[b] Fat as the sum of the fatty acids represents the sum of quantified individual fatty acid peaks (for which both certified and reference values are provided) as the triglycerides.

[c] The certified values for saturated, monounsaturated, and polyunsaturated fats are sums of the assigned values (certified and reference) for the individual fatty acids (as the fatty acids) in each group. The uncertainty is expressed as an expanded uncertainty, U, at the 95 % level of confidence, and is calculated according to the ISO Guide. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the measurement error. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriated associated degrees of freedom and 95 % confidence.

Table 2. Certified Concentration Values for Elements and Tocopherols[a]

	Mass Fraction (mg/kg)		
Calcium	411	±	18
Copper	4.93	±	0.15
Iron	16.4	±	0.8
Magnesium	1680	±	70
Manganese	16.0	±	0.6
Phosphorus	3378	±	92
Potassium	6070	±	200
Sodium	4890	±	140
Zinc	26.3	±	1.1
δ-Tocopherol	10	±	3
γ- + β-Tocopherol	100	±	19
α-Tocopherol	108	±	11

[a] Each certified concentration value, expressed as a mass fraction on an as-received basis, is the weighted mean of results provided by the laboratories listed in Appendix A and NIST. The uncertainty in the certified values, calculated according to the method described in the ISO and NIST Guides [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory, within-laboratory, and inhomogeneity components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. Analytical methodology information is provided in Appendix D.

Table 3. Reference Concentration Values for Proximates and Caloric Content[a]

	Mass Fraction (%)		
Solids	99.2	±	2.1
Ash	3.10	±	0.10
Protein	22.2	±	0.5
Carbohydrate (by difference)[b]	25.0	±	1.8
Total Dietary Fiber	5.57		0.42

	Calories
Caloric Content[c]	629 kcal/100 g ± 15 kcal/100 g

[a] Each reference concentration value, expressed as a mass fraction on an as-received basis, is the weighted mean of results provided by the laboratories listed in Appendix A. The uncertainty in the reference values, calculated according to the method described in the ISO and NIST Guides [2], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory, within-laboratory , and inhomogeneity components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. Analytical methodology information is provided in Appendix D. (The certified values for fat are provided in Table 1.)

[b] Based on fat as the sum of the fatty acids.

[c] The value for caloric content is the mean of individual caloric calculations from the laboratories listed in Appendix A. If the proximate values above are used for calculation, with caloric equivalents of 9, 4, and 4 for fat (as the sum of the fatty acids), protein, and carbohydrate, respectively, the mean caloric content is 637 kcal/100 g.

Table 4. Reference Concentration Values for Fatty Acids[a]

	Mass Fraction (%) as the Triglyceride	Mass Fraction (%) as the Fatty Acid
Heptadecanoic Acid (C17:0) (Margaric Acid)	0.050 ± 0.001	0.048 ± 0.001
Heptadecenoic Acid (C17:1)	0.035 ± 0.006	0.033 ± 0.006
Eicosadienoic Acid (C20:2)	0.017 ± 0.007	0.016 ± 0.007
(Z,Z,Z,Z)-5,8,11,14-Eicosatetraenoic Acid (C20:4 n-6) (Arachidonic Acid)	0.025 ± 0.016	0.024 ± 0.015
(Z)-13-Docosenoic Acid (C22:1 n-9) (Erucic Acid)	0.056 ± 0.012	0.054 ± 0.012

[a] Each reference concentration value, expressed as a mass fraction on an as-received basis, is the weighted mean of results provided by the laboratories listed in Appendix A. The uncertainty in the reference values, calculated according to the method described in the ISO and NIST Guides [2], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory, within-laboratory, and inhomogeneity components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. Analytical methodology information is provided in Appendix D.

Table 5. Reference Concentration Values for Amino Acids[a]

	Mass Fraction (%)
Alanine	0.93 ± 0.10
Arginine	2.65 ± 0.31
Aspartic Acid	2.83 ± 0.19
Cystine	0.27 ± 0.01
Glutamic Acid	4.69 ± 0.26
Glycine	1.41 ± 0.12
Histidine	0.55 ± 0.06
Isoleucine	0.77 ± 0.07
Leucine	1.56 ± 0.09
Lysine	0.78 ± 0.08
Methionine	0.21 ± 0.04
Phenylalanine	1.21 ± 0.08
Proline	0.96 ± 0.08
Serine	1.16 ± 0.09
Threonine	0.54 ± 0.08
Tryptophan	0.21 ± 0.06
Tyrosine	0.81 ± 0.14
Valine	0.94 ± 0.09

[a] Each reference concentration value, expressed as a mass fraction on an as-received basis, is the weighted mean of results provided by the laboratories listed in Appendix A. The uncertainty in the reference values, calculated according to the method described in the ISO and NIST Guides [2], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory, within-laboratory, and inhomomgeneity components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. Analytical methodology information is provided in Appendix D.

Table 6. Reference Concentration Values for Selected Water-Soluble Vitamins[a]

	Mass Fraction (mg/kg)		
Niacin	142	±	6
Pantothenic Acid	10.8	±	3.2
Vitamin B_1 Hydrochloride	0.84	±	0.17
Vitamin B_6	4.66	±	0.62

[a] Each reference concentration value, expressed as a mass fraction on an as-received basis, is the weighted mean of results provided by the laboratories listed in Appendix A. The uncertainty in the reference values, calculated according to the method described in the ISO and NIST Guides [2], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory, within-laboratory, and inhomogeneity components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. Analytical methodology information is provided in Appendix D.

Table 7. Reference Concentration Values for Aflatoxins and Acrylamide[a]

	Mass Fraction (ng/g)		
Aflatoxin B1	4.2	±	0.9
Aflatoxin B2	0.7	±	0.3
Total Aflatoxins[b]	5.0	±	0.5
Acrylamide[c]	87.0	±	7.8

[a] Each reference concentration value, expressed as a mass fraction on an as-received basis, is the weighted mean of results provided by the laboratories listed in Appendices A and B. The uncertainty in the reference values, calculated according to the method described in the ISO and NIST Guides [2], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory, within-laboratory, and inhomogeneity components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. Analytical methodology information is provided in Appendix D.

[b] The reference value for total aflatoxins is the mean of the laboratory means of the sum of aflatoxins Bl and B2.

[c] Each reference concentration value, expressed as a mass fraction on an as-received basis, is the weighted mean of results provided by the laboratories listed in Appendices A and C. The uncertainty in the reference values, calculated according to the method described in the ISO and NIST Guides [2], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory, within-laboratory, and inhomogeneity components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. Analytical methodology information is provided in Appendix D.

REFERENCES

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Certificate Revision History: 29 September 2004 (This revision reflects the addition of a reference value for acrylamide); 14 March 2003

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-6776; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.

APPENDIX A

The laboratories listed below performed measurements that contributed to the value assignment of SRM 2387 Peanut Butter.

Beech-Nut Nutrition Corporation; Canajoharie, NY, USA
Campbell Soup Company; Camden, NJ, USA
Covance, Inc.; Madison, WI, USA
General Mills, Inc.; Golden Valley, MN, USA
Hormel Foods Corporation; Austin, MN, USA
Kraft Foods, Inc.; Glenview, IL, USA
Krueger Food Laboratories, Inc.; Cambridge, MA, USA
Nabisco, Inc.; East Hanover, NJ, USA
Nestlé Food Corporation; Dublin, OH, USA
Novartis Nutrition Technical Center; St. Louis Park, MN, USA
Ralston Purina Company; St. Louis, MO, USA
U.S. Department of Agriculture, Food Composition Laboratory; Beltsville, MD, USA
Woodson-Tenent Labs; Memphis, TN, USA

APPENDIX B

The laboratories listed below performed aflatoxin measurements that contributed to the value assignment of SRM 2387 Peanut Butter.

Food and Drug Administration; Atlanta, GA, USA
Neogen Corporation; Lansing, MI, USA
U.S. Department of Agriculture, Agricultural Marketing Service; Blakely, GA, USA
Trilogy Analytical Laboratory; Washington, MO, USA

APPENDIX C

The JIFSAN Acrylamide Working Group laboratories listed below performed acrylamide measurements that contributed to the value assignment of SRM 2387 Peanut Butter.

American Oil Chemists Society, Champaign, IL USA
Covance Laboratories, Madison, WI, USA
Eurofins Scientific, Memphis, TN, USA
Federal Institute for Risk Assessment (BfR), Berlin, Germany
Food and Drug Administration (FDA), Center for Food Safety and Applied Nutrition, College Park, MD, USA
FDA, Office of Regulatory Affairs, Lenexa, KS, USA
Food Research Institute, University of Wisconsin, Madison, WI, USA
General Mills, Inc., Minneappolis, MN, USA
Health Canada, Ottawa, ON, Canada
Joint Research Centre, IRMM, Geel, Belgium
Livsmedelsverket (National Food Administration), Helsinki, Finland
National Food Processors Association, Washington, DC, USA
Nestlé, Lausanne, Switzerland
Procter and Gamble, Cincinatti, OH, USA
Swiss Quality Testing Services, Dietikon, Switzerland
The National Food Laboratory, Dublin, CA, USA

APPENDIX D

The methodological information reported by laboratories whose results were used for value assignments is summarized below. The number of laboratories using a particular method is provided in parentheses.

Proximates, Fatty Acids, Total Dietary Fiber, Amino Acids, and Calories

Solids	Moisture determined by mass loss after oven-drying: Forced-air oven (3) Vacuum oven (8)
Ash	Mass loss after ignition in muffle furnace (11)
Extractable Fat	Acid digestion, ether extraction (2) Chloroform/methanol extraction (1) Soxhlet ether extraction (1) Pressurized-fluid extraction (NIST)
Fatty Acids	Hydrolysis followed by gas chromatography (11) Pressurized-fluid extraction (hexane/acetone) followed by gas chromatography (GC; NIST)
Nitrogen	Kjeldahl (7) Thermal conductivity (1) Pyrolysis, GC (1) Pyrolysis, conductivity (1) Dumas combustion (1)
Protein	Calculated; a factor of 5.46 was used to calculate protein from nitrogen results
Carbohydrate	Calculated; solids - (protein + fat as the sum of fatty acids + ash)
Total Dietary Fiber	Enzymatic – gravimetry (8)
Amino Acids	Hydrolysis, derivatization, liquid chromatography (LC; 5)
Calories	Calculated; 9(fat) + 4(protein) + 4(carbohydrate)

Vitamins

α-Tocopherol	Saponification – reversed-phase liquid chromatography (RPLC) – fluorescence detection (3 + NIST) Saponification – normal-phase liquid chromatography (NPLC) – absorbance detection (1) Saponification – NPLC – fluorescence detection (3)
δ- and γ- + β-Tocopherol	Saponification – RPLC – fluorescence detection (1 + NIST) Saponification – NPLC – absorbance detection (1) Saponification – NPLC – fluorescence detection (3)
Niacin	Microbiological (7)
Pantothenic Acid	Microbiological (6)
Total Vitamin B_1 Hydrochloride	Digestion – fluorescence detection (4) Extraction – RPLC – fluorescence detection (1) Extraction – ion pair chromatography – fluorescence detection (1)

Total Vitamin B_6	LC – fluorescence detection (1) Microbiological (5)

Elements

Calcium	Flame atomic absorption spectrometry (1) Direct current plasma atomic emission spectrometry (1) Inductively coupled plasma optical emission spectrometry (10 + NIST)
Copper	Flame atomic absorption spectrometry (2) Direct current plasma atomic emission spectrometry (1) Inductively coupled plasma optical emission spectrometry (8 + NIST)
Iron	Flame atomic absorption spectrometry (1) Direct current plasma atomic emission spectrometry (1) Inductively coupled plasma optical emission spectrometry (10 + NIST)
Magnesium	Flame atomic absorption spectrometry (1) Direct current plasma atomic emission spectrometry (1) Inductively coupled plasma optical emission spectrometry (10 + NIST)
Manganese	Flame atomic absorption spectrometry (1) Direct current plasma atomic emission spectrometry (1) Inductively coupled plasma optical emission spectrometry (9 + NIST)
Phosphorus	Absorption spectrophotometry (3) Inductively coupled plasma optical emission spectrometry (9 + NIST)
Potassium	Flame atomic absorption spectrometry (1) Direct current plasma atomic emission spectrometry (1) Inductively coupled plasma optical emission spectrometry (9 + NIST)
Sodium	Flame atomic absorption spectrometry (1) Direct current plasma atomic emission spectrometry (1) Inductively coupled plasma optical emission spectrometry (9 + NIST)
Zinc	Flame atomic absorption spectrometry (1) Direct current plasma atomic emission spectrometry (1) Inductively coupled plasma optical emission spectrometry (10 + NIST)

Other Analytes

Aflatoxins	LC – fluorescence detection (6) Thin-layer chromatography (1) Enzyme-linked immunosorbent assay (1)
Acrylamide	LC-mass spectrometry (MS; 1) LC- tandem mass spectrometry (MS/MS) (18) GC-MS (2) GC-MS/MS (1)